Conflict of interest statement
None declared.

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One-step immunochromatographic assay for screening of coeliac disease

Luis Sorell, Jose A Garrote, Boris Acevedo, Eduardo Arranz

Tissue transglutaminase is the autoantigen that elicits endomysial antibodies, which are the serological hallmarks of coeliac disease. We describe a simple, rapid immunochromatographic assay for IgA and IgG antibodies to transglutaminase, which is highly accurate for diagnosis of this disease. Results were positive for all samples from 50 untreated coeliac patients, and negative for 40 non-coeliac patients with gastrointestinal disorders. The assay seems to be a useful alternative to biopsy for mass screening for coeliac disease.

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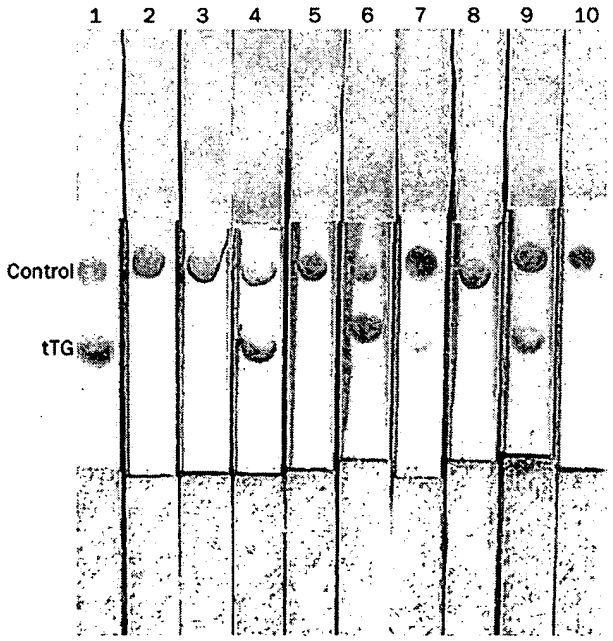
Coeliac disease is one of the most common food-sensitive enteropathies in man. Although the definitive diagnosis of coeliac disease is based on characteristic histological changes seen in jejunal biopsy specimens, serological tests, such as the detection of circulating antibodies to gliadin and to endomysium, are cheaper, less invasive methods of screening for this disease.

Since 1997, when Dietrich and co-workers identified tissue transglutaminase (tTG) as the major antigen recognised by antibodies to endomysium,[1] different ELISAs have been developed for the detection of autoantibodies to tTG as predictors of coeliac disease.[2] Balders and colleagues reported a rapid dot blot assay for the detection of antibodies to transglutaminase.[3] We describe a one-step immunochromatographic assay for the detection of both IgA and IgG antibodies to transglutaminase in human serum or plasma.

1 μL of guineapig tTG (Sigma, T-5398, lot 99H7425), at 1·5 g/L in 50 mmol/L Tris buffered saline with 5 mmol/L $CaCl_2$ pH 7·5, was adsorbed onto one end of a plastic-backed nitrocellulose membrane strip (pore size 10 μm) to form a reactive zone. A polycationic substance that binds tTG-colloidal gold conjugates was adsorbed onto the same membrane to form a control zone adjacent to the reactive zone. tTG was conjugated to colloidal gold particles and dried onto an inert fibrous support (type CNPF-S1-L2-P50, Advances Microdevices PVT Ltd, Ambala Cannt, 133001, India) which was attached to the plastic backing of the nitrocellulose strip, to achieve minimum direct contact with the beginning of the nitrocellulose membrane, close to the reactive zone. In this system, when the conjugate support is dipped in serum or plasma, any antibodies to tTG in the sample react with the colloidal-gold tTG, developing an immunocomplex that migrates through the membrane strip. The immobilised tTG in the nitrocellulose reacts with the immunocomplexes, forming a coloured dot in the reactive zone. Excess of conjugate and immunocomplexes continue migration and finally react with the control reagent, forming a second coloured dot on the strip. A positive result, indicating the presence of antibodies to tTG in the sample, is seen as two dots on the strip, with a pink-to-purple color. A negative assay shows only a control dot (figure). Results are obtained in less than 10 minutes.

Serum or plasma samples were obtained from patients and controls after informed consent. Two independent observers who were unaware of the patients' diagnosis made the test readings. There was full agreement between the two.

The immunochromatographic assay results were positive for all samples from 50 untreated patients with coeliac disease diagnosed on the basis of the revised European Society of Paediatric Gastroenterology And Nutrition criteria for coeliac disease,[4] for a sensitivity of 100% (95% CI, 92·9–100%). The same samples were tested for IgA antibodies to transglutaminase with a commercial ELISA (Celikey,



Detection of antibodies to tissue transglutaminase by immunochromatographic assay
1, 4, 6, 7, and 9: positive samples; 2, 3, 5, 8, and 10: negative samples.

Pharmacia & Upjohn, Freiburg, Germany), and for IgA antibodies to monkey endomysium by indirect immunofluorescense (BioSystem, Barcelona, Spain). 47 of the 50 samples were positive by ELISA, for a sensitivity of 94% (88·2–98·4%), and 48 were positive by indirect immunofluorescence, for a sensitivity of 96% (89·1–99%). All samples from 40 non-coeliac patients with gastrointestinal disorders were negative by the three assays (immunochromatographic assay, ELISA and indirect immunofluorescence), for a specificity of 100% (91·2–100%). Sensitivity and specificity of all the assays were calculated with the result of small-bowel biopsy as gold standard. One patient with coeliac disease had a deficit of IgA and therefore a negative result by ELISA and indirect immunofluorescence.

We tested again for antibodies to tTG in 15 patients with normal intestinal histology on gluten-free diet, or after gluten challenge, and found one false positive with our assay, and five false positives with ELISA. In six patients with partial villous atrophy (three on gluten-free diet and three after gluten challenge), the immunochromatographic assay showed three false negatives, as opposed to four false negatives with the ELISA. Finally, in six patients with evident intestinal injury (total or subtotal villous atrophy) after gluten challenge, one false negative appeared with the ELISA (patient with IgA deficit) but none with the immunochromatographic assay. Additionally, our assay was positive in two out of 21 first degree relatives of patients with coeliac disease without symptoms. These two individuals also had a positive result by ELISA and by indirect immunofluorescence assay, suggesting that they might have a clinically silent form of the disease, which would require biopsy to confirm diagnosis. The cost of each test is less than US$1.

This immunochromatographic assay detects antibodies to transglutaminase quickly and easily. It is highly accurate in detection of untreated patients with coeliac disease, and detects both IgA and IgG antibodies to transglutaminase, which prevents misdiagnosis of patients with a deficit of IgA, a frequent trait of coeliac disease.[5] The assay can be done in a doctor's surgery, and seems to be a useful alternative way to screen for coeliac disease, especially in patients with few or with untypical symptoms, and in risk groups—ie, people with insulin dependent diabetes, Down's syndrome, autoimmune diseases, and first degree relatives of patients with coeliac disease.

Contributors

L Sorrell and B Acevedo developed the immunochromatographic assay. J A Garrotte and E Arranz selected the patients. L Sorrell and J A Garrotte did tests by immunochromatography and ELISA. J A Garrotte did immunofluorescence. All authors contributed to the analysis and reporting of results.

Conflict of interest statement

None declared.

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Autologous thrombin for treatment of pseudoaneurysms

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Femoral pseudoaneurysms arise in up to 2% of patients after femoral cannulation for cardiac catheterisation. We used autologous thrombin for percutaneous obliteration of pseudoaneurysms occurring after catheterisation. We prepared autologous thrombin isolates from blood of ten patients with femoral pseudoaneurysms, and injected this solution into the pseudoaneurysms with duplex imaging guidance. We then assayed thrombin activity. All pseudoaneurysms were successfully thrombosed without substantial complications, although three patients needed a repeat procedure within 24 h. We have shown that autologous thrombin-induced thrombosis of pseudoaneurysms is reliable, simple, safe, and cheaper than commercial bovine or human thrombin, and avoids risks of anaphylaxis and contamination with prions.

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Femoral pseudoaneurysms arise in up to 2% of patients after cardiac catheterisation, and risk factors include large diameter catheters, failure to puncture over the femoral head, inadequate compression after sheath removal, and abnormal haemostasis including that induced by heparin and warfarin treatment. Some pseudoaneurysms expand rapidly, and urgent surgery is needed; others (usually <2 cm maximum diameter) resolve if managed conservatively. Most pseudoaneurysms fall between these two extremes, cause local symptoms of pressure and pain, fail to resolve, and therefore need intervention.

Ultrasound-guided compression repair of pseudoaneurysms has largely been replaced by percutaneous obliteration with commercial thrombin kits. The largest reported series is that of Kang and colleagues,[1] in which 82 of 83 pseudoaneurysms were treated with thrombin kits without surgery, with only minor complications. Use of the thrombin kit for this reason is not licensed, and long-term safety is unknown; anaphylaxis has been reported.[2,3]

Because the thrombin kits are a product of non-recombinant DNA, there is a potential risk of prion transmission. Isolation of autologous thrombin, and use of this substance to induce venous thrombosis in vivo, has been described.[4] We adapted the technique and applied it to the treatment of arterial pseudoaneurysms.

We recruited patients with symptomatic pseudoaneurysms greater than 2 cm in diameter. We excluded those with rapidly expanding pseudoaneurysms, groin sepsis, or who had received heparin infusions or warfarin treatment. Six males and four females, mean age 64 years (SD 12·0), had undergone femoral catheterisation 3–120 days (median 5) before presentation. Apart from one puncture with an 8 F trocar, all patients had 7 F punctures. All patients gave informed consent and the Wandsworth local research ethics committee approved the study.

We did the 1-h extraction of thrombin at room temperature with a class II hood facility. We took 30–60 mL of blood from